Physical Mapping of Rice Chromosomes 8 and 9 with YAC Clones

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Abstract
First efforts for physical mapping of rice chromosomes 8 and 9 were carried out by ordering YAC clones of a rice genomic DNA library covering six genome equivalents with mapped DNA markers. A total of 79 and 74 markers from chromosomes 8 and 9, respectively, were analyzed by YAC colony and Southern hybridization using RFLP markers of cDNA and genomic clones, and by polymerase chain reaction (PCR) screening using PCR-derived and sequence-tagged site (STS) markers. As a result, 252 YAC clones were confirmed to contain the mapped DNA fragments on both chromosomes. A contig map was constructed by ordering these YAC clones and about 53% and 43% genome coverage was obtained for chromosomes 8 and 9, respectively, assuming a YAC clone size of 350 kb and overlap between neighboring YACs of 50%. A continuous array of YAC clones with minimum overlap gave a total size of 18.9 Mb for chromosome 8 and 15.6 Mb for chromosome 9, which are close to previous estimates. These contig maps may provide valuable information that can be useful in understanding chromosome structure and isolating specific genes by map-based cloning.

Key words: physical map; chromosome 8; chromosome 9; YAC contigs; YAC islands

1. Introduction
One of the major challenges of rice genetics is mapping of the whole rice genome so that breeders can identify and manipulate genes of agronomical importance. At the Rice Genome Research Program (RGP), we have established a high-resolution rice genetic map which is the densest map available in plants that is backed up with sequence data.1 With a total of 1,383 DNA markers, this map has already been proven to be useful in understanding rice genome structure such as the distribution of single- and multi-copy genes,1,2 conserved regions within the genome3 as well as the synteny with other cereal crops.4,5,6,7 Furthermore, all information derived from this map could be applied to other mapping populations of rice with great accuracy.8 At present, we are using this map to construct an array of overlapping cloned genomic fragments, or contigs, covering the 12 rice chromosomes, and ultimately to reconstruct the whole rice genome. Towards this goal, a yeast artificial chromosome (YAC) rice genomic DNA library of about 7,000 clones with an average insert size of 350 kb and covering six genome equivalents has been constructed from a japonica rice variety, Nipponbare, one of the parents of the mapping population used for our high-density genetic map.9 So far, this library has been successfully used to establish contig maps covering more than half of rice chromosomes 1, 5 and 6 by the chromosome landing technique.10,11,12 Similarly, physical maps for the other chromosomes are being constructed in order to understand thoroughly rice genome organization and function and to provide important tools for genetic manipulation of rice and other cereal crops.

In this paper, we report on our first efforts for physical map construction of rice chromosomes 8 and 9. In our high-density linkage map, a total of 79 and 74 DNA markers, including a number of expressed sequence tags (ESTs), have been mapped to chromosomes 8 and 9, respectively. Several morphological and biochemical markers such as sug (sugary endosperm), Dn-1 (dense panicle 1) and lam(t) (low-amylose endosperm) have also been localized in the classical genetic map of these chromosomes,13 Among the many disease and insect resistance genes, Xa-t, a bacterial blight resistance gene,14 Pi-11(t), a Pyricularia oryzae resistance gene15 and gm, a gall midge resistance gene,16 have also been located...
on these chromosomes. Therefore, contig maps covering the whole of chromosomes 8 and 9 will be very useful in clarifying the genomic organization of these chromosomes, and more importantly, such maps will be useful in the isolation of various localized genes by map-based cloning.

2. Materials and Methods

2.1. DNA markers and YAC library

A total of 79 markers from chromosome 8 and 74 markers from chromosome 9, which were previously mapped in our high-density restriction fragment length polymorphism (RFLP) linkage map of rice, were used.1 These markers consisted of cDNA clones from callus and root, genomic clones, YAC-end clones, NotI linking clones, wheat clones as well as PCR-derived markers including randomly amplified polymorphic DNAs (RAPDs) and sequence-tagged site (STS) clones.17,18,19 The data and information for all markers in our linkage map are available on the World Wide Web (WWW) at http://bank.dna.afrc.go.jp.

The YAC library used for chromosome landing was derived from genomic DNA of a japonica rice variety, Nipponbare. The details of the construction and maintenance of our YAC library were previously described by Umehara et al.8 This library consisted of 6,932 YAC clones blotted on five high-density filters and multiplied on many replica filters. The YAC filters and independent clones are also available from our DNA bank and are accessible at the web site mentioned above.

2.2. Screening of YAC library

Initially, the YAC genomic library was screened using cDNA clones, genomic clones and other RFLP clones mapped in both chromosomes by colony hybridization. The replica filters blotted with YAC clones were hybridized with 10 ng/ml of the probe by enhanced chemiluminescence (ECL) hybridization system (Amersham). YAC clones which hybridized with the probe were selected and yeast DNAs were extracted by the cell lysis method, separated by pulsed-field gel electrophoresis (PFGE) and blotted on nylon membranes. Then subsequent hybridization with pYAC1 left-arm-specific sequence was done using the ECL hybridization system. YAC sizing was performed using the GelReader V.2 computer software.

2.3. YAC sizing

The insert size of positive YAC clones was determined as previously described9,21 with some modifications. Intact yeast chromosomal DNAs were isolated by the cell lysis method, separated by pulsed-field gel electrophoresis (PFGE) and blotted on nylon membranes. Then subsequent hybridization with pYAC1 left-arm-specific sequence was done using the ECL hybridization system. YAC sizing was performed using the GelReader V.2 computer software.

2.4. Contig map construction

All YAC clones with the confirmed fragment of the mapped DNA marker were arrayed in conjunction with the RFLP linkage map of rice. Further confirmations using YAC clones selected for neighboring markers were done to establish a contiguous array of YAC clones covering as many markers as possible. In the case of locus positions with multiple markers, the order was determined based on the arrangement of YACs carrying the specific marker fragments.

3. Results

3.1. YAC selection with mapped DNA fragments

Although 79 and 74 DNA markers have been mapped on chromosomes 8 and 9, respectively, only 71 DNA markers in chromosome 8 and 61 markers in chromosome 9 were able to identify YAC clones from our rice genomic YAC library. A total of 21 markers appeared to be either unsuitable for YAC screening or could not select any YAC clones in our library. Ten such markers were Kasalath (an indica rice variety used as the other parent in genetic mapping) dominant markers in which the corresponding allelic band in Nipponbare could not be confirmed. In addition, two DNA markers on chromosome 8 and nine DNA markers on chromosome 9 did not hybridize with any of the YAC clones in our library.

From the combined results of colony/Southern hybridization and PCR screening, we were able to select 386 YAC clones (187 for chromosome 8 and 199 for chromosome 9) from our YAC library with about 7,000 clones which contained at least one of the mapped DNA marker fragments. Many of these YAC clones hybridized with two or more DNA markers. In chromosome 8, a total of 27 YACs were confirmed to carry 2 DNA marker fragments and 8 YACs with 3 or more marker fragments. In particular, Y3711, Y4319, Y1362 and Y6684 were able to detect four markers each from chromosome 8. On
chromosome 9, a total of 39 YACs were found to carry two DNA marker fragments and 20 YACs with three or more marker fragments. Such clones as Y2756, Y6676 and Y6983 could identify as many as 5 DNA markers each. Thus, 131 unique YAC clones were ordered by 71 DNA markers on chromosome 8 and 121 unique YACs in the case of 61 markers on chromosome 9.

Several chimeric clones which were assigned by two or more different markers on different chromosomes or positions were obtained for both chromosomes. A total of 14 YACs on chromosome 8 and 22 YACs on chromosome 9 were chimeric, representing 11% and 18% of the total YACs selected for each chromosome, respectively. In addition, several YAC clones possessing unmapped copies of the marker sequences particularly using multi-copy markers were also identified in Southern analysis. Twenty-six YAC clones selected for markers on chromosome 8 and 30 YAC clones on chromosome 9 contained unmapped copies of multi-copy markers.

3.2. Chromosome 8 contig map

The contig map for chromosome 8 is shown in Fig. 1. All 131 unique YACs were aligned with 71 DNA markers along the entire length of the chromosome. Some of these YACs could not exactly identify the mapped DNA fragment but were ordered just the same because these fragments co-exist with those already mapped on chromosome 8. These YACs selected by co-existing fragments are shown in the figure as bars with a square in the middle to differentiate them from YACs selected by mapped DNA fragments which are represented by bars with circles and included Y2432 (selected for C83), Y1848 (R2382), Y1658 and Y5631 (C411) among others. In addition, a few YACs which showed deletion were also selected. These are shown in the figure by YACs with a gray bar in the middle to represent the deleted segment. As an example, the end marker C83, and another marker, R662, separated by 1.8 cM were identified in Y4946 but two markers in-between were not identified.

A total of eight contigs with YACs that identified two or more markers were obtained for 32 markers. These YAC contigs covered a genetic distance of 17.2 cM and appear as dark regions on the genetic map shown in Fig. 1. The longest contig was obtained for the region from R2662 to G56, covering five DNA markers with a genetic distance of 4.1 cM. The rest of the contigs covered a range of 0.3 cM to 3.6 cM. In the case of the remaining 29 DNA markers, YAC islands with only one YAC at each marker position were obtained for 11 markers and YAC islands with multiple YACs at a single marker position for 18 markers. A continuous array of YAC clones with minimum overlaps covering the entire chromosome is shown by bars with filled circles in Fig. 1. This minimum tiling path consisted of 46 YACs with sizes ranging from 60 kb to 810 kb. The simple sum of these minimal YAC overlaps could cover a total of about 18.3 Mb of chromosome 8.

Estimation of the chromosome coverage of ordered YACs for chromosome 8 is summarized in Table 1. The total physical distance covered by YAC contigs and YAC islands was determined assuming an average YAC clone size of 350 kb9 and overlap between neighboring YACs which means 350 kb x 1.5 = 525 kb for one marker position. A total of eight YAC contigs selected by 32 DNA markers covered a physical distance of 4.7 Mb. On the other hand, the 29 YAC islands with a single YAC and multiple YACs at single marker positions covered a total of 3.8 Mb and 9.4 Mb, respectively. Thus, the total coverage of all anchored YACs was estimated to be 17.9 Mb which corresponds to 53% of the entire length of chromosome 8.

3.3. Chromosome 9 contig map

The contig map of chromosome 9 has a total of 121 YACs which consisted of aligned YACs on the mapped DNA markers as well as those containing copies which co-exist with mapped fragments (Fig. 2). These anchored YACs formed nine YAC contigs and 30 YAC islands. The longest contigs were obtained from P134 to R3312 with a genetic distance of 5.8 cM and from C506 to L984 with a genetic distance of 3.5 cM. The YAC islands which consisted of only one YAC at individual marker positions were obtained for five DNA markers whereas YAC islands with multiple YACs at single marker positions were obtained for 12 markers. The minimum tiling path consisted of 44 YAC clones with insert sizes ranging from

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<th>Table 1. Chromosome coverage of YAC contigs and YAC islands for chromosomes 8 and 9 of rice.</th>
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<td>Chr. 8</td>
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<tr>
<td>Genetic distance (cM)</td>
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<td>Chromosome length (Mb)*</td>
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<td>(a) YAC contigs</td>
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<td>(b) YAC islands with single YAC**</td>
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<td>(c) YAC islands with multiple YACs***</td>
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<td>Physical distance (Mb)</td>
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<td>Total physical distance covered by YACs</td>
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<td>Chromosome coverage****</td>
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* The genetic map of rice has a total distance of 1,575 cM and the genome size is 430 Mb, therefore 1 cM is 273 kb.
** Based on an average YAC size of 350 kb.
*** Based on an average YAC size of 350 kb and assuming overlap of about 50% between neighboring YACs which means 350 kb x 1.5 = 525 kb for one marker position.
**** Chromosome coverage: Physical distance (a + b + c) /chromosome length.
**Figure 1.** A physical map of rice chromosome 8 constructed by ordering a YAC rice genomic library using RFLP and STS markers from a high-density linkage map of rice. A total of 79 markers have been mapped to chromosome 8 but only 71 markers could identify YAC clones in our library. The genetic map and the physical map, which consists of YAC contigs and YAC islands, are shown as regions 1 and 2 with the genetic position of the top and bottom markers. The dark portions in the genetic map represent the genetic distance covered by YAC contigs. The DNA markers with selected YACs are listed to the left of the genetic map. The YAC clones that carry the mapped fragment represented by bars with open and filled circles are aligned with the markers. Those with filled circles were arbitrarily selected to form a minimum tiling path and the corresponding insert size is indicated on top of each YAC. The rest of the YACs selected for each marker are shown as bars with open circles. YAC clones with letter (C) following the numbers are chimeric YACs. Markers that could not select any YAC clone from our library are indicated on the right side of the genetic map with Kasalath-dominant markers shown in underlined letters.
Figure 2. A physical map of rice chromosome 9 constructed by ordering a YAC rice genomic library using RFLP and STS markers from a high-density linkage map of rice. A total of 74 DNA markers have been localized in the genetic map of chromosome 9 but only 61 markers could identify YAC clones in our library. The legends as well as the marker notations are as described in Fig. 1. A YAC-end clone mapped in this chromosome is shown as a bar with filled square in the physical map.
260 to 1130 kb, for a total of about 15.6 Mb.

All 74 DNA markers in chromosome 9 were localized to 47 locus positions and a total of 39 markers were mapped at 13 locus positions, with each locus containing two or three DNA markers tightly linked at 0 cM. At the distal region of chromosome 9, a total of 12 markers were clustered at one locus position next to the uppermost end marker. Eighteen YACs were identified carrying the ten DNA markers at this locus (L710 and R265B could not select any YACs). The minimum tiling path for this region shows that this single locus position on the genetic map may extend up to about 1.8 Mb in length.

The exact position of several markers has been clarified from the contig map. The locus positions of two PCR-derived markers, namely, P107 at the upper region and T4 at the lower region of chromosome 9 have been clarified based on the order of YACs selected for these markers as well as the neighboring markers. Thus, P107 is located above G1017 and R1164 whereas T4 is located below G293 and L984. This has also been confirmed by re-mapping these markers together with additional DNA markers in our second generation genetic map (data not shown).

The estimated chromosome coverage of ordered YACs for chromosome 9 is also summarized in Table 1. The total physical distance covered by YAC contigs and YAC islands were also determined based on the 350 kb average size of YAC clones in our library and on the 50% overlap between neighboring YACs. A total of nine contigs selected by 29 DNA markers gave an estimated physical distance of 3.5 Mb whereas the 17 YAC islands with single and multiple YACs on single marker positions showed an estimated physical length of 8.4 Mb. Thus, the total coverage of all anchored YACs was estimated to be 11.9 Mb which corresponds to 43% of the entire length of the chromosome.

The physical maps for chromosomes 8 and 9 together with all information on the DNA markers and YAC clones are accessible on the World Wide Web at http://bank.dna.affrc.go.jp.

4. Discussion

The contig maps of chromosomes 8 and 9 presented here consist of YAC contigs and YAC islands covering nearly half of the genetic map for each chromosome. Similar maps for other chromosomes of rice previously reported or to be reported soon showed that almost half of each chromosome is covered by ordered YAC clones. The estimates of 17.9 Mb and 11.9 Mb for chromosomes 8 and 9, respectively, are based on two assumptions. First, that the average insert size of YAC clones in our library with about 7,000 YAC clones is approximately 350 kb, and second, that overlap between neighboring YACs is 50%. However the actual value of YAC overlap may vary from one YAC to another among neighboring YACs. Furthermore, the number of YACs identified for each marker may invariably influence the chromosome coverage, particularly for single position YACs. Thus, unless a contig is established for markers in adjacent loci, the actual physical distance may significantly differ from the estimated values. Another measure of the physical distance covered by ordered YAC clones, that is, the simple sum of the DNA inserts of YACs which constitute the minimum tiling path and therefore constitute a continuous array of ordered minimum overlap YACs covering the entire chromosome, was used in this study. For YAC clones which comprise the minimum tiling path, total physical lengths of 18.3 Mb and 15.6 Mb were obtained for chromosomes 8 and 9, respectively. These values are very close to the estimated physical distance, suggesting that we have already obtained a reliable estimate of the physical length of both chromosomes.

Physical mapping could prove useful in clarifying some aspects of the genome structure of specific regions of the chromosome. On chromosome 9, almost half of the markers were localized at the same locus positions as other markers. In most cases, two or more markers at the same locus position were identified in separate YAC clones as in the case of ten DNA markers with different fingerprints mapped at the distal end of chromosome 9. Several YACs were identified which carried 2-5 markers mapped in this locus position and with insert sizes ranging from 300 kb to 460 kb. Thus, the actual physical length of loci with multiple markers could be accurately determined. Furthermore, dissection of loci with two or more markers by arranging YAC clones may also provide valuable information that may be important in the positional cloning of genes localized in such regions. Another feature that may be revealed by physical mapping is the physical order of markers in such regions with recombination. Based on the order of multiple selected YACs, the exact physical position of markers that form clusters at the same locus on the genetic map could be resolved. For markers which could not be accurately mapped, as in the case of markers P107 and T4 on chromosome 9, physical mapping could also provide an alternative and reliable approach for determining their exact position on the chromosome.

Chimerism, internal rearrangements and deletions have always been associated with YAC cloning. In the present study, an average of about 15% of the selected YACs were chimeric. A few YAC clones were also found to contain deletions. However, these aberrations were not considered significant because most of the markers are ordered with a minimum of two YAC clones. Therefore, YACs without chimerism, deletions or rearrangements, which constitute a majority of ordered YACs, could provide a reliable framework in defining specific regions of the chromosomes.

Our YAC library did not yield YAC clones for all
the markers tested. A total of 11 DNA markers could not identify any clones in the YAC rice genomic library. Physical mapping in other rice chromosomes also revealed specific regions which are not represented in our library.10,11,12 These regions could correspond to chromosomal segments which are difficult to clone in yeast vectors and/or may be regions containing repeated sequences.24 Furthermore, although YAC clones have been selected for most of the markers in our genetic map, overlapping YAC clones that form contigs could be ordered in only a few regions of both chromosomes 8 and 9. About 70% of the physical length covered by YACs corresponded to YAC islands with single or multiple YACs at single marker positions. Thus, it may be necessary to determine the extent of overlaps among these YAC islands as well as YAC contigs by strategies other than chromosome landing. In addition, several regions in the map, as long as 8.5 cM in chromosome 8 and 15.6 cM in chromosome 9, contained no DNA markers. We are trying to fill these gaps in the map by adding more markers, although these regions may correspond to regions with low recombination frequency, to similar chromosomal regions which show no polymorphism between crossed parents, or to highly repetitive DNA sequences which are difficult to map with our strategy. Further analysis by physical mapping may reveal characteristic features of these regions of the chromosomes.

In the next stage of the construction of a physical map of chromosomes 8 and 9, we will use additional DNA markers which have been recently mapped in our high-density linkage map. Completion of a physical map will also require other strategies such as DNA fingerprinting to confirm YAC overlaps and chromosome walking to fill the gaps between contigs. This may provide a reliable framework of specific regions of the chromosomes that will be necessary for map-based cloning. Eventually, a physical map covering the entire rice genome will make available a vast amount of useful information for isolation and utilization of important genes in rice and other crops.

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